Bioremediation Approaches for Sustained Uranium Immobilization Independent of Nitrate Reduction

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Abstract

The daunting prospect of complete nitrate removal at DOE sites such as the FRC provides strong incentive to explore bioremediation strategies that will allow for uranium bioreduction and stabilization in the presence of nitrate. Typical in-situ strategies involving the stimulation of metal reducing bacteria are hindered by the low pH environment and require that the persistent nitrate be continuously transformed. This project investigates the possibility of stimulating nitrate-indifferent pH-tolerant organisms to achieve non-specific bioreduction of U(VI) despite nitrate.

Enrichments from FRC Area 2 sediments were prepared using a variety of electron donors (ethanol, glycerol, hydrogen, and glycerol) and MOPS/TRIS buffers at pHs ranging from 4.9 to 7. Successful enrichments containing 10-20 mM methanol have demonstrated the nearly complete reduction of uranium (90% reduction at ~10 ppm) with very little loss of nitrate (less than 10% loss at ~850 ppm) from pH 4.9-5.5. Many higher pH enrichments also demonstrated similar U reduction capacity with 5-30% nitrate loss. Bacterial16S rRNA genes from successful enrichments at pH 5.7-6.7 were amplified and sequenced for phylogenetic analysis. A majority of clone sequences retrieved from enrichment cultures were comprised of Clostridia, Clostridia-like organisms and Bacteroidetes.

Further experiments tested the stability of ~2 ppm U(IV) in nitrate or nitrite solutions. When added to water with oxygen removal, U(IV) was stable and oxidized only when exposed to air. The presence of nitrite (100 ppm) or nitrate (1000 ppm) did not induce measurable oxidation over the several week timescale of measurements.

Nitrate-indifferent uranium reduction

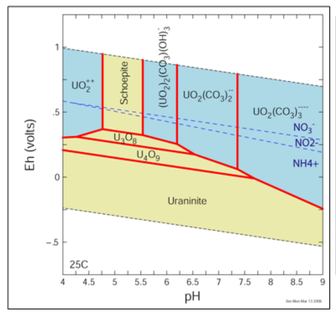

Fig. 1 – pH-E_h diagram of dominant nitrogen and uranium species typical of conditions used in this study (10 ppm U, 6.7% pCO_2). Both nitrate and nitrite are potential electron acceptors from U(IV) and may be reduced before U(IV).

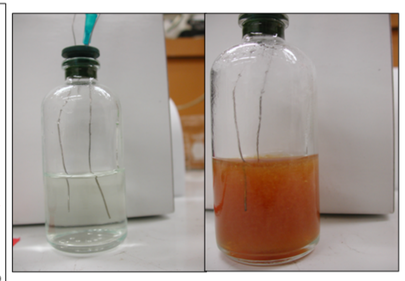

Fig. 2 – 1 mM U(VI) acetate before (a) and after (b) electrochemical reduction of solution to U(IV) solid in the presence of 1000 ppm sodium nitrate.

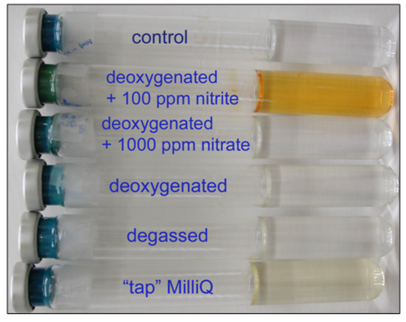

Fig. 3 – The stability of 0.9 mM Fe(II) in solutions of various levels of oxygen removal or containing nitrate or nitrite after 100 days. Only the "tap" MilliQ water and solution containing 100 ppm nitrite showed oxidation.

U(IV) stability experiments

U(IV) stability experiments were conducted by adding U(IV), Fe(II), or rezasurin to pressure tubes containing sterile MilliQ water. The water was either used directly, degassed, or deoxygenated, and various tubes containing nitrate (1000 ppm) or nitrite (100 ppm). In contrast with Fe(II) (Fig. 3), no U(IV) oxidation has been observed in either nitrate or nitrite solutions after 100 days.

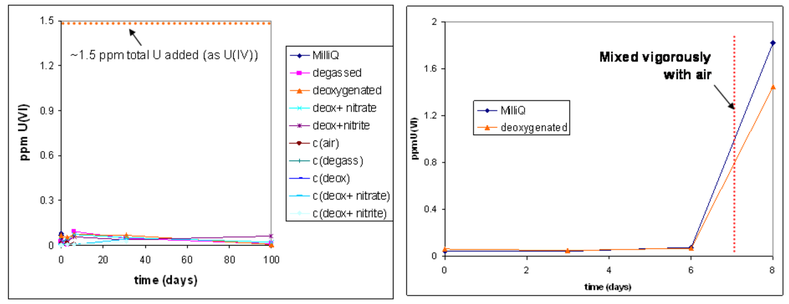

Figure 4 a) U(VI) measured by KPA and b) demonstrating complete oxidation when mixed with air. The label "c" designates a control sample.

FRC enrichments

Enrichments from ERSP Field Research Center sediments were prepared with a modified phosphate-buffered basal medium, an electron donor (H2, methanol, ethanol, or glycerol), MOPS/TRIS buffers at pHs between 4.9 and 6.2, NaNO3 (800-1000 ppm), and NaHCO3. Those enrichments exhibiting growth were transferred to fresh modified PBBM medium with added nitrate and uranium (VI) in sterile pressure tubes, again at various pHs with a series of electron donors. Bicarbonate was added in an amount consistent with FRC conditions (0.1-10 mM, depending on pH) .

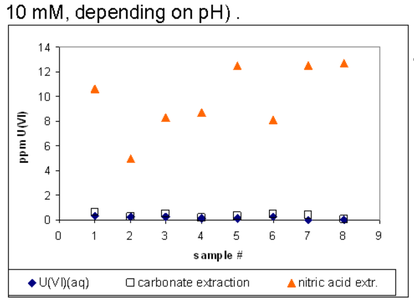

Figure 5. KPA measurements of filtered (aqueous U(VI)), 0.5 M bicarbonate extracted ("total" U(VI)), and concentrated nitric acid extracted (total U) uranium.

	Label	donor	pH	% loss NO3-	% loss U
1	YMM6ii	MeOH	6.2	13.7	99.0
2	YMM6i	MeOH	6.2	18.8	98.9
3	YMG6i	Glycerol	6.2	7.4	92.9
4	RMM6ii	MeOH	5.9	4.6	90.1
5	RMM6i	MeOH	5.9	8.2	98.4
6	RMG6i	Glycerol	5.9	7.6	91.8
7	BMM6ii	MeOH	5.7	22.1	98.2
8	BMM6i	MeOH	5.7	11.9	98.8
9	BMG6i	Glycerol	5.7	30.7	87.1
	YMG6ii	Glycerol	6.2	65.9	76.6

Table 1. Biologically mediated uranium loss from solution in the presence of nitrate in FRC enrichment cultures.

Enrichments from pH 5.7-6.2 were sampled including two extractions. Filtered samples for aqueous U(VI) and samples extracted with 0.5 M bicarbonate solution for additional sorbed or precipitated U(VI) were prepared in an anaerobic glovebag. Concentrated nitric acid extraction was performed under exposure to air, and ideally measures the total uranium content (Elias et al., J. Microbiol. Meth. 53 (2003) 343–353).

Enrichments prepared at pHs from 4.9-5.4 also demonstrated U loss with limited nitrate reduction (Table 2, right). Experiments are currently verifying the transferability of these enrichments.

Label	donor	pH	% loss NO3-	% loss U
GRMM6ii	MeOH	4.9	5.3	91.2
GBMM6i	MeOH	4.9	7.8	95.0
ORMM6ii	MeOH	5.4	5.6	93.3
OBMM6i	MeOH	5.4	0	94.0

Molecular characterization of enrichment cultures

16s rRNA analysis (Figure 6) of clone sequences retrieved from these enrichments indicates that

- Clostridia and Clostridia-related organisms comprise the largest clusters within the tree
- Cluster A contains sequences from all samples
- Cluster B sequences primarily from the aerobic gram negative genera *Haemophilus* and *Aeromonas*
- Cluster C clone sequences are from the Bacteroidetes

Label	donor	pH*	Clone sequence Cluster(s)
YMM6i	MeOH	6.2	A, C
YMM6ii	MeOH	6.2	A, B, C
YMG6i	glycerol	6.2	A, B
RMM6i	MeOH	5.9	A
RMM6ii	MeOH	5.9	A
RMG6i	glycerol	5.9	A
BMM6i	MeOH	5.7	A, B, C
BMM6ii	MeOH	5.7	A

Table 3. Clone sequence clusters refer to Fig. 6

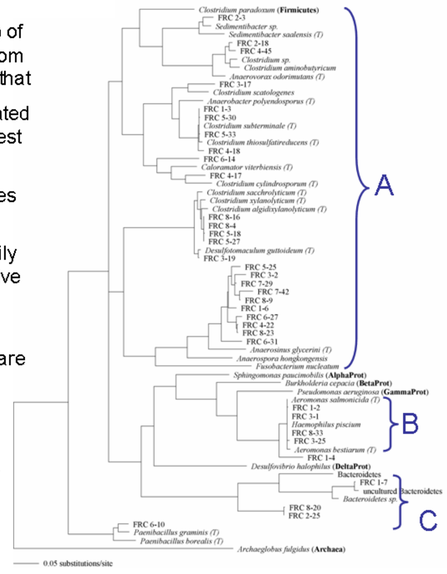

Fig. 6 – Genetic distances among bacterial species were used to construct a phylogenetic tree using the maximum- likelihood method.

Summary and Implications

- This research is investigating innovative biotransformation strategies to provide long-term stability of immobilized metals and radionuclides without requiring complete nitrate removal in low pH environments
- The presence of nitrite or nitrate did not induce measurable U(IV) oxidation over the timescale of several months
- Enrichments from FRC sediments have demonstrated the nearly complete reduction of uranium (~90% reduction at ~10 ppm) with very little loss of nitrate from pH 4.9-6.2
- A majority of clone sequences retrieved from enrichment cultures were comprised of Clostridia, Clostridia-like organisms and Bacteroidetes
- *As long as anaerobic conditions are maintained, this research demonstrates the potential for uranium reduction without complete and consistent nitrate removal through the activity of organisms such as the Clostridia, even if only a small fraction of the total reducing equivalents are directed toward uranium reduction.*

Acknowledgements

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